REMARKS

The subject invention relates to a new method of using laser light to treat the tissue. Laser energy, including light from an Nd:YAG laser, has been used for tissue treatment for quite some time. For example, Nd:YAG laser energy has been used to treat vascular lesions and for hair removal. Most often, the Nd:YAG laser was configured to generate relatively long pulses, in the millisecond regime. Nd:YAG lasers have also been operated in the Q-switched mode, typically generating pulses in the nanosecond regime, for procedures such as tattoo removal. By contrast, there has been less investigative work using Nd:YAG lasers with pulse widths in the microsecond regime.

The inventors herein have developed a procedure for an Nd:YAG laser that has been used to successfully treat certain tissue problems, such as skin redness. This approach uses microsecond laser pulses, each pulse having a very high energy or power. As recited in new claim 103, each pulse should have a power of at least 10 kilowatts. Preferably, each pulse should have a power of as much as 40 kilowatts.

As best described in the specification at page 19, line 26 to page 20 line 23, in the method of the subject invention, the handpiece which is used for delivering the laser pulses is spaced away from the tissue at a distance of a few centimeters. As can be appreciated, since the handpiece is spaced from the tissue, there is no surface cooling as is used in many prior applications. The handpiece is moved back and forth over the area to be treated, typically a few square centimeters. During the time the handpiece is moved over the target region, a series of laser pulses are delivered to the tissue. In accordance with claim 103, at least 400 of these high power pulses are delivered. This treatment has proved effective at reducing the redness in tissue and has shown some success in reducing wrinkles.

Attached as Exhibit A and B are two papers discussing clinical investigations related to the claimed procedure. Exhibit A is "*Nonablative Facial Remodeling*," Schmults, Arch. Dermatology, Vol. 140, November 2004, page 1371 (2004). Exhibit B is "*Assessment of the Efficacy of Nonablative Long-pulsed 1064-nm Nd:YAG Laser Treatment of Wrinkles Compared at 2, 4 and 6 Months*," Trelles, Facial Plastic Surgery, Vol. 21, No 2, (2005).

The article of Exhibit A relates to a treatment for reducing skin redness or "erythema." A CoolGuide Vantage system from the assignee was used in the treatment. The Vantage system includes an Nd:YAG laser generating pulses at a 1.06 micron wavelength. As described on the

front page of the article, each patient was treated with laser pulses having a fluence of 13J/cm^2 with a pulse duration of 300 microseconds. The handpiece was spaced from the tissue on the order of 2 to 4 centimeters and no cooling was performed. The tissue was treated in four sections with a combined total of 12,000 to 14,700 pulses of light. The user continuously moved the handpiece in a back and forth fashion. This preliminary study indicated that the treatment can reduce redness and stimulate collagen formation which improves skin quality.

The article of Exhibit B relates to the treatment of wrinkles. A Vantage laser system of the type described above was used. As noted on page 147, the researchers used a pulse width of 300 microseconds and a fluence of 13 J/cm^2. The handpiece was held about 2 centimeters away from the skin and was scanned from left to right and top to bottom until 500 pulses were delivered. This preliminary study showed reduction in wrinkles for at least some of the patients.

Applicants wish to the note that at least the study in described in the article of Exhibit A was funded in part by the assignee as is common when new devices and procedures are being developed. It should also be noted that each of these studies were published in peer reviewed journals.

In response to the Final Office Action, and in order to expedite prosecution, Applicants have cancelled all of the pending claims and have submitted a single new independent claim and a set of dependent claims targeted to applicants' preferred method of operating a Nd:YAG pulsed laser system. It is believed that this preferred system is not taught or suggested in the prior art of record.

Turning to the Office Action, the Examiner had rejected the previously pending claims based on Eckhouse (5,776,175). Eckhouse primarily relates to a flashlamp based light source used to treat tumors. The background section of the Eckhouse patent discusses the problems with X-ray, microwave and laser based treatments. In particular, Eckhouse notes that Nd:YAG lasers are not suitable for tumor treatment because of their small spot size. In contrast, Eckhouse teaches that for treating tumors, relatively large spot sizes, several square centimeters, are desirable.

Eckhouse then teaches that in his preferred embodiment, the radiation source should be a flashlamp 14 which emits broadband, incoherent radiation. Eckhouse discloses a number of very broad ranges of operating parameters. For example, the treatment spot can range from 0.8 cm^2 up to 500 cm^2. The pulse width can range from 100 microseconds to 50 milliseconds. Energy

densities as low as 100 millijoules/cm^2 are disclosed (column 5, line 3) while energy densities as high as 120 J/cm^2 are disclosed (column 2, line 20 and line 37). These suggested fluences cover a range of three orders of magnitude.

Eckhouse fails to teach or suggest the method of new independent claim 103. As noted above, Eckhouse teaches away from the use of narrowband radiation from an Nd:YAG. None of the pulse width, energy or spot size parameters described by Eckhouse are associated with a laser system, only his flashlamp system. Furthermore, nowhere does Eckhouse teach or suggest a method which relies on a series of high power laser pulses, each pulse greater than 10 kilowatts. While it is no doubt true that a combination of the highest fluences suggested by Eckhouse (such as 120J/cm^2) with his shortest pulses would produce high power pulses, nowhere does Eckhouse teach that such an approach is helpful or required. Regardless, and as noted above, Eckhouse's parameters are associated with the operation of a broadband flashlamp, not a narrowband laser.

Beyond the differences noted above, Eckhouse does not teach or suggest a method wherein a handpiece is provided that is spaced from the tissue during treatment. Eckhouse is silent on this issue. Eckhouse describes a quartz light guide 22 for delivering the radiation to the treatment site or emitting light directly from an opening in the housing (column 4, line 66). Eckhouse also fails to disclose a treatment method wherein a handpiece is moved back and forth over the skin while delivering at least 400 pulses of laser light to the areas to be treated. Rather than scanning the handpiece to treat a region larger than the spot size, Eckhouse teaches that the spot size should be increased. "By applying the radiation over a larger area, for example, 500 cm^2, even heating of large tumors can be achieved, reducing the chance of uneven tumor treatment and the risk of damaging tissue." (column 5, line 39)

Based on the above, it is respectfully submitted that new independent claim 103 is not anticipated or rendered obvious by Eckhouse and allowance thereof, along with the claims depending therefrom is respectfully solicited.

Respectfully submitted,

STALLMAN & POLLOCK LLP

Dated: December 17, 2008

By: _____
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EXHIBIT A

OBSERVATION

Nonablative Facial Remodeling

Erythema Reduction and Histologic Evidence of New Collagen Formation Using a 300-Microsecond 1064-nm Nd:YAG Laser

Chrys D. Schmults, MD; Robert Phelps, MD; David J. Goldberg, MD

Background: A variety of nonablative lasers have been used to improve skin color and toning. Evidence of new collagen has been seen. Using blinded observer analysis of electron microscopic changes, we have documented the effect of a nonablative Nd:YAG laser on collagen production and its relationship to patient age.

Observations: Ultrastructural analysis of 9 patients showed a decrease in overall collagen fiber diameter in the papillary dermis at 1 month and 3 months after 3 treatment sessions. This is consistent with the formation of new collagen. Younger patients had a greater decrease in collagen fiber diameter compared with older patients. The change in collagen fiber diameter with time as well as the relationship between that change and the patient's age were statistically significant ($P<.001$). Photographic evaluation showed that those patients with preexisting erythema showed improvement in erythema along with an associated improvement in skin quality. There were no adverse events.

Conclusions: Microsecond Nd:YAG lasers appear to be safe for nonablative laser remodeling. Our study indicates that microsecond Nd:YAG lasers can produce new collagen formation in the papillary dermis. In addition, the condition of patients with erythema may be improved. Younger patients may form more new collagen compared with older patients with photodamage.

Arch Dermatol. 2004;140:1373-1376

BOTH NANOSECOND (Q-switched) and millisecond (long-pulsed) Nd:YAG lasers are currently used for nonablative dermal remodeling.[1-7] They are thought to stimulate new collagen production by producing a thermal injury to the dermis that initiates a wound-healing response.[8] During wound healing, procollagen and type III collagen fibers are produced initially and have a small diameter. Later in the wound-healing process, thicker type I collagen fibers are made and cross-linking occurs, leading to an increase in the average diameter of collagen fibers in the dermis.[9] Collagen fiber diameter can be measured via electron microscopy (EM). Electron microscopy studies of nonablative lasers such as the 585-nm flashlamp pulsed dye laser have shown a decrease in diameter of dermal collagen fibers after nonablative laser therapy.[10] A decrease in collagen fiber diameter has been associated with production of new collagen,[8,9] which is thought to increase skin firmness and improve skin texture in patients after treatment.

Recently, intermediate pulsed Nd:YAG lasers have been developed with pulse durations in the microsecond range. We investigated one of these new microsecond Nd:YAG lasers to determine its safety and efficacy in nonablative dermal remodeling by using clinical photographs and EM analysis of dermal collagen fibers.

METHODS

Ten women aged 28 to 67 years with erythema and/or fine lines were enrolled in a study approved by the Pascack Valley Hospital (Westwood, NJ) institutional review committee. Subjects had Fitzpatrick skin types I through III. After signing a consent form, all subjects' faces (excluding the periorbital area) and jawline were treated 3 times at 2-week intervals with a 1064-nm Nd:YAG laser (CoolGlide Vantage; Cutera, Brisbane, Calif).

See also pages 1333 and 1379

Prior to treatment, the skin was cleansed with a standard bacteriostatic soap and water. Subjects' eyes were protected with stainless steel external ocular shields, and laser pulses were applied on the skin adjacent to but outside the orbital rim. The laser parameters were set to a fluence of 13 J/cm^2, a pulse duration of 300 microseconds, and a spot size of 5 mm. A smooth, rapid painting motion was used to administer treatment, with the tip of the instrument 2 to 4 cm above the skin surface. Based on this separation from the skin, the actual laser beam at the skin surface is 6 to 7 mm in diameter, with corresponding fluences from 9 to 7 J/cm^2, respectively. No cooling was performed.

Author Affiliations: Skin Laser and Surgery Specialists of New York and New Jersey, New York and Hackensack (Drs Schmults and Goldberg); Department of Dermatology, University of Pennsylvania, Philadelphia (Dr Schmults); and Department of Dermatology, Mount Sinai School of Medicine, New York (Dr Phelps).

Financial Disclosure: None.

Figure 1. A, Erythema of the face before treatment with a 300-microsecond 1064-nm Nd:YAG laser; B, 3 months after final treatment.

Figure 2. A, Erythema of the face before treatment with a 300-microsecond 1064-nm Nd:YAG laser; B, 3 months after final treatment.

The face was treated in 4 sections, with a combined total of 12000 to 14700 light pulses applied per treatment. The pulses were applied at a rate of 7 Hz with a continuous motion of the handpiece at approximately 8 cm/s such that the pulses had little, if any, overlap. This was accomplished through a back and forth motion in 1 direction, combined with gradual motion in the perpendicular direction. Within each section of the face, repeated passes were made. The end point was determined based on the number of pulses. Each entire treatment consisted of approximately 30 minutes.

Two-millimeter punch biopsy specimens were obtained at baseline and at 1 and 3 months after final treatment. Biopsy specimens were taken from the infra-auricular sun-exposed area. Sequential biopsy specimens were taken nearly adjacent to each other. Collagen fibers from the papillary dermis were visualized and photographed via EM by previously described methods.[11,12] Collagen fiber diameter of longitudinally cut fibers was measured directly on the photographs in millimeters, then adjusted for magnification to give a diameter in nanometers. For each biopsy specimen, a blinded physician observer measured and recorded the diameter of collagen fibers from 5 photographs. Ten fibers were measured per photo for a total of 50 collagen fiber measurements per biopsy specimen. The measurements for each specimen were averaged and then a mean fiber diameter for all patients was calculated for each time point.

Statistical analysis of the collagen fiber measurements was performed using SAS, version 8.2 (SAS Inc, Cary, NC), on a UNIX platform. The fiber diameter reduction from baseline to 1 and 3 months was tested using the 1-sided paired *t* test. Linear regression analyses were performed, and Pearson correlation coefficients were calculated for the correlations between age and change in fiber size from baseline to 1 and 3 months. An analysis of variance (ANOVA) was performed to analyze the relationship between the fiber size distributions and the time point.

In addition to the ultrastructural measurements, subjects were photographed at baseline, prior to each treatment, and at 1 and 3 months after final treatment. Digital photography with identical lighting conditions was used for all photographs. Photographs were evaluated by 2 nonblinded physicians, and changes in erythema and skin quality were assessed at each time point as compared with baseline. At each visit, patients were assessed for adverse effects, including erythema, edema, purpura, blistering, pigmentary changes, and scarring. At each visit, all subjects were asked if they had any adverse events between visits.

RESULTS

There were no adverse events reported in this study. Treatments were well tolerated by all patients, with minimal discomfort. Transient erythema occurred after almost all treatments but disappeared within hours of treatment.

One patient was lost to follow-up after the first treatment and was excluded from all analysis. One patient was excluded from clinical analysis owing to photographic errors but was still included in the EM collagen fiber analysis.

Four patients had noticeable erythema prior to treatment. All 4 of these patients had a reduction in erythema at 1- and 3-month follow-up visits as evidenced by pho-

Table. Mean Diameters of Collagen Fibers

Patient No./Age, y	Baseline Fiber Diameter, nm	1-mo Diameter, nm	3-mo Diameter, nm	Change (Decrease) in Diameter, nm	
				At 1 mo*	At 3 mo†
1/52	60.7	60.0	55.0	0.8	5.8
2/35	71.9	61.5	64.7	10.4	7.2
4/58	47.8	47.8	51.7	<0.1	−3.9
5/31	77.6	60.2	53.6	17.4	24.0
6/44	53.9	62.8	49.8	−9.0	4.1
7/67	55.6	62.8	66.1	−7.2	−10.5
8/32	67.2	56.8	53.2	10.4	14.1
9/43	62.3	55.7	52.6	6.6	9.7
10/38	67.6	52.7	52.5	14.9	15.2
Mean/40	62.7	57.8	55.4	4.9	7.3

*P=.08
†P=.03

tographic evaluation. The improvement was greatest at 3 months. Skin texture was also improved in areas of erythema reduction as evidenced by photographic evaluation (**Figure 1** and **Figure 2**).

Mean collagen fiber diameter decreased compared with baseline at 1 and 3 months after the final treatment (**Table**) (**Figure 3**) in 7 of 9 patients. This difference approached statistical significance at 1 month (P=.08) and was significant at 3 months (P=.03). The relationship between fiber width distribution and the 3 time points (Figure 3) was significant (P=.02) by the ANOVA. An example set of EM photographs demonstrating the decrease in fiber size is shown in **Figure 4**.

The decrease in mean collagen fiber diameter appeared to be related to age. Younger patients appeared to have a greater decrease in collagen fiber diameter at 3 months (**Figure 5**). The linear regression analysis showed that for regression between subject age and change in fiber size from baseline to 1 month, the P value is .01 (Pearson correlation coefficient, −0.78), and to 3 months the P value is <.001 (Pearson correlation coefficient, −0.91).

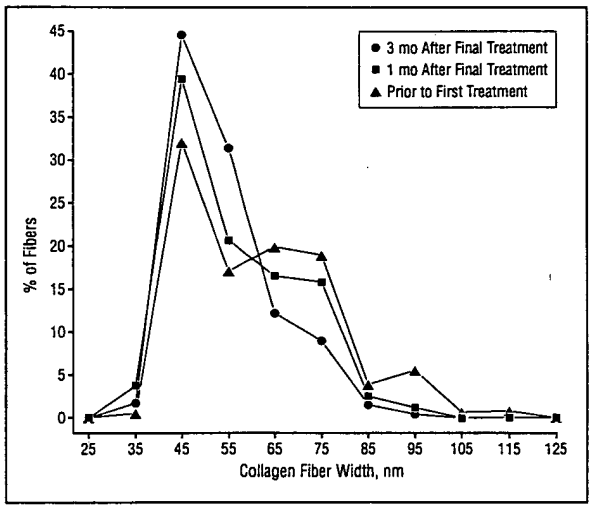

Figure 3. Mean percentage of collagen fibers of varying sizes before and 1 and 3 months after treatment.

COMMENT

The microsecond Nd:YAG laser investigated in this study appears to be safe for Fitzpatrick skin types I through III when used within the parameters studied and may be useful for reducing facial erythema and improving skin texture. The significant decrease in collagen fiber diameter seen by EM analysis at 3 months after treatment suggests that new collagen is being produced.[8,9] This is consistent with an early wound-healing response in which thin procollagen and/or collagen III fiber production is increased in the papillary dermis. This new collagen production may be responsible for the improvement in skin quality seen after nonablative treatments.

Older patients appeared to have little to no decrease in collagen fiber diameter after treatment. This may suggest that older patients do not produce new collagen in response to nonablative laser therapy. The oldest patient in this study (patient 7: age, 67 years) actually showed an increase in collagen fiber diameter. Marked solar elastosis was present microscopically in all 3 biopsy specimens from this patient. Elastosis can impede accurate measurement of collagen fiber diameters, making data analysis difficult to interpret by our methods. When the data for patients 58 years and younger (n=8) were analyzed, the significance of the decrease in mean collagen fiber diameter was more evident with baseline mean diameter of 63.6 nm, 1-month diameter of 57.2 nm, and 3-month diameter of 54.0 nm. The difference between baseline and 1-month diameters (6.4 nm) was statistically significant (P=.04), as was the difference between baseline and 3-month diameters (9.5 nm) (P=.007).

It is possible that elastosis can block laser energy from hitting its targets and thereby inhibit its effectiveness in stimulating dermal remodeling. This may be why the 2 oldest patients in our study did not show a decrease in collagen fiber diameter. Older patients with photodamage may have minimal new collagen production in response to therapy. Anecdotal reports from clinicians indicate that younger patients with less photoaging have the greatest improvement from nonablative laser therapies. This study in-

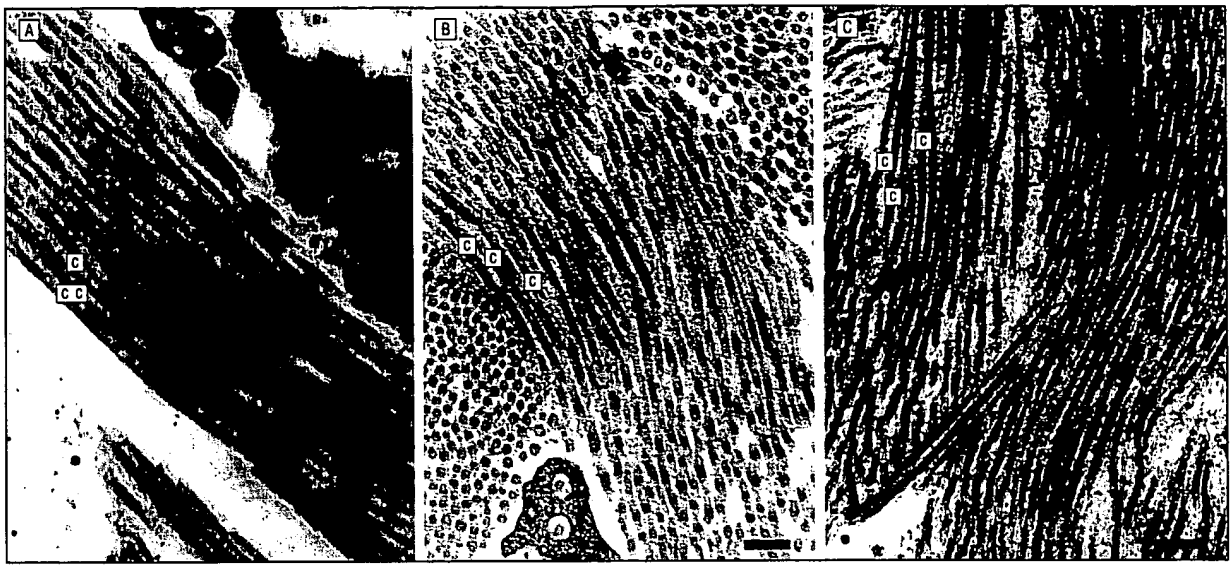

Figure 4. Electron micrographs of collagen fibers in the papillary dermis in 1 patient (A, pretreatment; B, 1 month after treatment; and C, 3 months after treatment [reticulated background represents either proteoglycans or glycosoaminoglycans]). The collagen fibers show typical periodicity and are cut longitudinally and transversely. There is gradual decrease in average width from pretreatment to 3 months (scale bar, 320 nm; original magnification ×40000). Typical collagen fibers are labeled "C."

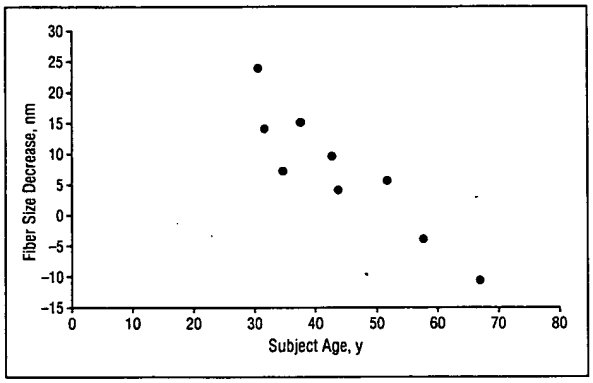

Figure 5. Mean decrease in collagen fiber diameter 3 months after final treatment.

dicates that microsecond Nd:YAG lasers can reduce facial erythema and improve skin texture. The skin textural changes may be similar to those reported with a variety of nonablative lasers.[13-16] A statistically significant reduction in collagen fiber diameter after treatment indicates formation of new collagen. This may be 1 mechanism by which microsecond Nd:YAG lasers exert their clinical effects. Older patients with photodamage had less of a reduction in collagen fiber diameter. This may reflect less new collagen formation, which may lead to reduced clinical effects in such patients.

Further work remains to determine the role that age and photodamage may play in dermal remodeling and clinical effectiveness after nonablative laser resurfacing. Studies are also needed to better define the mechanisms by which nonablative laser resurfacing exerts effects on collagen and other components of the dermal matrix.

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EXHIBIT B

Assessment of the Efficacy of Nonablative Long-Pulsed 1064-nm Nd:YAG Laser Treatment of Wrinkles Compared at 2, 4, and 6 Months

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ABSTRACT

Rhytides represent an aesthetic problem for a large percentage of the population. Many methods, both noninvasive and invasive, have been used for the treatment of wrinkles. Recently, the long-pulsed 1064-nm Nd:YAG laser has been shown to enhance dermal collagen synthesis without damaging the epidermis. The purpose of this preliminary study is to evaluate the use of the long-pulsed Nd:YAG laser in the nonablative treatment of periocular and perioral wrinkles. Ten patients with facial wrinkles were treated with the long-pulsed 1064-nm Nd:YAG laser, at a spot size of 5 mm in diameter, energy density of 13 J/cm^2, exposure time per pulse of 300 microseconds, and a repetition rate of 7 Hz. All patients had a total of three treatments, once every 2 weeks. Subjective (patient satisfaction index [SI]) and objective (both physician- and computer program-based clinical index [CI]) assessments were performed before the first and third treatment sessions, and at 2, 4, and 6 months after the last treatment. At 6 months after the final treatment session, the patients' subjective SI was maintained at 40%, and had peaked at 50% 2 months after the final session. Physician assessment showed a CI of 40% at the 6-month assessment point and the computer program showed a 50% CI. The greatest level of effect with long-pulsed Nd:YAG laser nonablative skin rejuvenation for facial wrinkles was seen 2 months after the final treatment. Effects were still visible at the 6-month period, but showed a tendency to decrease. Maintenance treatments are required to achieve good patient satisfaction.

KEYWORDS: Long-pulsed Nd:YAG laser, perioral wrinkles, periocular wrinkles, nonablative skin rejuvenation, neocollagenesis

Lasers: New Technology and Emerging Trends; Editors in Chief, Fred Fedok, M.D., Gilbert J. Nolst Trenite, M.D., Ph.D., Daniel G. Becker, M.D., Roberta Gausas, M.D.; Guest Editor, Paul J. Carniol, M.D., F.A.C.S. *Facial Plastic Surgery*, Volume 21, Number 2, 2005. Address for correspondence and reprint requests: Mario A. Trelles, M.D., Ph.D., Instituto Médico Vilafortuny/Antoni De Gimbernat Foundation, Av. Vilafortuny 31, E-43850 Cambrils, Spain. [1]Instituto Médico Vilafortuny/Antoni De Gimbernat Foundation, Cambrils, Spain; [2]Computer Architecture Department, University of Málaga, Málaga, Spain; [3]Centre Laser Dermatologique, Marseille, France. 0736-6825,p;2005,21,02,145;153,frx,en; fps00546x.

Many different methods have been used in the treatment of facial wrinkles, including chemical and mechanical peels, fillers, botulinum toxin, and light energy of various wavelengths and wavebands with lasers and intense pulsed light systems. The basic concept behind the use of the laser in the treatment of wrinkles is the induced formation of new dermal collagen following a deliberate deposition in the mid to upper dermis of laser energy-induced thermal damage due to the absorption of the laser energy in the target tissue.[1,2] The neocollagenesis induced by laser energy may follow laser skin ablation (including total removal of the epidermis) or nonablative skin rejuvenation (in which the photothermal damage in the dermis is delivered through an intact epidermis). It is generally accepted that the lasers of choice in ablative resurfacing are the Er:YAG and the CO_2 lasers, or a combination of both in the one system.[3–5] To deposit enough residual thermal damage in the dermis, these lasers must first remove the epidermis. Ablative laser resurfacing is associated with excellent results in the removal of even deep wrinkles and the provision of a very good skin condition, but these are offset by lengthy patient downtime as a result of postoperative crusting, edema, and prolonged erythema, which may also be complicated by infection, scarring, and herpes-related lesions.[6,7] The nonablative approach allows the deposition of thermal damage to the dermis, followed by neocollagenesis, but without damaging the epidermis and without any downtime. However, a variety of supplementary and complementary treatment methods are required to get good results, and even these are inferior to the efficacy of the ablative approach.[8–10]

The long-pulsed Nd:YAG laser at the wavelength of 1064 nm might permit the deposition of a good packet of energy in the dermis, in a nonablative manner, thereby provoking a degree of irritative dermal photothermal damage that is sufficient to induce the various stages of the wound healing process, finally resulting in the formation and modeling of new collagen. Epidermal protection with this type of system is often accomplished with a cryocooling device incorporated in the laser handpiece. This study was designed to assess the efficacy of a long-pulsed Nd:YAG system in the treatment of perioral and periocular wrinkles, with postoperative assessments at 2, 4, and 6 months.

SUBJECTS AND METHODS

Patients

The study subjects comprised 10 patients (seven females and three males), skin types ranging from II to IV, (four type II, four type IV, and two type II), and ages ranging from 36 to 61 years (mean age, 47.2 ± 8.85 years). Patients had wrinkles in a variety of sites (perioral, six patients; periocular, three patients; and forehead, one patient), wrinkle grades I to III (Table 1). Following an explanation of the trial and its aims and objectives, all patients gave their written informed consent. The study was approved by the Ethics Committee of the Antoni de Gimbernat Foundation. No patient had received treatment of the face with any kind of preparation prior to the trial. Each patient received three treatments separated by 2-week intervals.

Laser System and Treatment

The system used in the study was the Altus Vantage (Cutera Inc., San Francisco, CA), a long-pulsed Nd:YAG system emitting at 1064 nm. The handpiece can deliver a spot size of from 3 to 10 mm in diameter, and depending on the spot size selected, the system automatically programs the other treatment parameters such as the pulse duration, energy, and the repetition rate

Table 1 Details of Patients and the Efficacy at the 6-Month Assessment

				Wrinkles		Results		
Patient No.	Gender	Age	Phototype	Grade	Site	Subj*	Obj†	Comp‡
1	F	38	III	II	POc	1	2	2
2	F	47	III	II	POc	2	2	2
3	F	39	II	II	POc	2	3	3
4	F	65	IV	III	POr	2	3	3
5	M	42	III	I	POc	1	3	3
6	F	36	II	II	POr	3	2	3
7	F	58	II	III	POc	3	3	3
8	M	44	II	I	POc	3	2	2
9	M	61	III	III	Brow	2	2	2
10	F	52	IV	II	POr	1	2	2

*Grades: 1 = not satisfied; 2 = fairly satisfied; 3 = satisfied; 4 = very satisfied.
†Grades: 1 = 0–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100% improvement.
‡Grades: 1 = 100–76%; 2 = 75–51%; 3 = 50–26%; and 4 = 25–0% improvement.
POc, periocular; POr, perioral; Subj, patient satisfaction; Obj, clinician assessment from clinical photography; Comp, computer system.

of each pulse. We used a spot size of 5 mm in diameter, an energy density (radiant flux) of 13 J/cm^2, pulse width of 300 microseconds, and a repetition rate of 7 Hz. No pretreatment anesthesia was required. The patients wore appropriate protective eyewear. In accordance with the manufacturer's instructions, the treatment head was held approximately 2 cm away from the skin and scanned smoothly over the area to be treated, scanning both from left to right and top to bottom, "painting" the entire area to be treated, until a total of approximately 500 pulses was completed for each treatment area. If the patient complained of pain, then the handpiece was moved slightly further from the tissue and scanning was done a little faster.

Subjective Patient Assessment

The patient assessment was performed using questionnaires, in which the patients were asked to give their degree of satisfaction based on a 5-point scale from 0 to 4 (0 = worse; 1 = little satisfaction or not satisfied; 2 = fairly satisfied; 3 = satisfied; and 4 = very satisfied). Questionnaires were given out after the third session, and then at 2, 4, and 6 months thereafter.

Assessment of Pain and Erythema

The degree of pain during treatment was assessed by the patients on a 4-point scale from 1 to 4 (1 = no pain; 2 = some pain; 3 = very painful, but bearable; and 4 = unbearable pain. Erythema related with the treatment was assessed just after treatment and in the 48 hours after treatment on a 4-point scale (+ = no erythema; ++ = some erythema; +++ = noticeable erythema; and ++++ = intense erythema).

Histological Assessment

To examine the dermal morphology associated with our treatment technique, our patients volunteered for histological specimen biopsies (patients 1, 5, 7, and 8); all biopsies were taken from the preauricular area. Biopsies were taken before the first treatment session and 2 months after the final treatment session; specimens were placed in formalin and routinely processed for hematoxylin and eosin staining.

Clinical Photography

Objective clinician assessment was based on the clinical macrophotography of the treated area with the patient's face at rest before the first and after the third treatment session, then at 2, 4, and 6 months after the third and final session. For treatment of the forehead, the patient was asked to maintain a relaxed, neutral posture of the face with the eyes open. For the periorbital area, the patient remained at rest with his or her eyes gently closed. For patients who were having the perioral area treated, during the photography they were asked to keep their mouth closed, without exerting any force, in as natural a position as possible. The photography was performed in the same room, with the patient in the same position under the same ambient light source. Digital photography was captured with the Sony Mavica MVC-FD91 (2 megapixels, high-resolution setting) (Sony Corp., Tokyo, Japan). The clinician assessment was on a 5-point scale from 0 to 4: 0 = worse; 1 = little or no improvement (0 to 25%); 2 = some improvement (26 to 50%); 3 = good improvement (51 to 75%), and 4 = excellent improvement (76 to 100%). Assessment was performed by an independent blinded expert.

Computer Evaluation

The objective physician evaluation from the clinical photography was complemented with a computerized automated image extraction system, which was capable of assessing wrinkle depth and wrinkle direction. Samples from the clinical photography were automatically normalized by removing noise, standardizing brightness and scaling, and adjusting contrast and luminosity parameters. All of these procedures were performed by the computer program. This software was developed together with the Department of Computer Architecture, the University of Málaga (Spain).[11]

A Canny operator was next used as an optimal edge detector, working in a multistage process, which resulted in an image made up of 1-pixel-thick connected segments that closely followed the faint margins of the wrinkles.[12] In this way, the pre- and posttreatment conditions and areas of the wrinkles in each image were computed, and improvements were calculated based on a 100% baseline of the images taken before the first treatment, then compared after the third treatment, and then at 2, 4, and 6 months thereafter. The improvements in percentages were assigned to a 5-point scale where 0 = worse, 1 = little or no improvement (100 to 74%); 2 = fair improvement (75 to 51%); 3 = good improvement (50 to 26%); and 4 = very good improvement (25 to 0%).

RESULTS

All 10 patients completed the study. At the 6-month final assessment point, 30% (three of 10) of the patients were not satisfied with the result, 30% (three of 10) were fairly satisfied, and 40% (four of 10) were satisfied. No patient was very satisfied, and none got worse. The satisfaction index (SI) was calculated by adding the number of patients in grades 3 and 4 (satisfied and very satisfied) expressed as a percentage of all patients; thus the SI was 40%. The physician assessment placed

Table 2 Evaluations of Postoperative Pain and Erythema,* Hyper- and Hypopigmentation, and Other Complications

	Reactions		Side Effects and Complications			
Patient No.	Pain†	Erythema‡	Burn	Hyperpigmentation	Hypopigmentation	Others
1	1	+	—	—	—	—
2	3	++	—	—	—	—
3	1	+	—	—	—	—
4	1	+	—	—	—	—
5	1	+	—	—	—	—
6	2	++	—	—	—	—
7	1	+	—	—	—	—
8	1	+	—	—	—	—
9	3	+++	—	—	—	—
10	1	+	—	—	—	—

*Totally evolved in all patients in 24–48 hours.
†Pain scale: 4 = unbearable; 3 = very painful; 2 = painful; 1 = little or no pain.
‡Erythema scale: ++++ = intense; +++ = noticeable; ++ = some; + = little or none.

six of the 10 patients (60%) in the fair improvement group and the remaining four patients (40%) in the good improvement group. The subjective clinical index (CI) was calculated by adding the patients in the good and very good groups only; thus, the physician CI was 40%. The computer system findings had 50% in the fair improvement and 50% in the good improvement group; thus, the computer CI was 50%. In neither of the objective evaluations did any patient get worse, and no patient was scored in the very good improvement group (Table 1).

Regarding pain, two of the 10 patients communicated that treatment was very painful but the pain was bearable, one patient had some pain, and the remaining six patients had no pain. In all patients, erythema spontaneously resolved in 24 to 48 hours. One patient scored noticeable erythema, three patients scored some erythema, and the remaining six patients scored little or no erythema. Neither pain nor erythema was related with the patient skin phototype. There were no side effects or other complications seen in any of the patients (Table 2).

Despite the comparatively poor findings in patient satisfaction at the 6-month point, with only 40% of the patients satisfied, there was an interesting peak in the patient SI at the 2-month assessment point up to 50%, which then decreased between the 2- and 4-month points and leveled out at 40%, better than that after the third and last treatment session of approximately 30%. Roughly the same distribution and 2-month peak in the CI was seen in both the physician and computer assessments (Fig. 1).

Fig. 2A shows the average patient assessment of the improvement in the depth of wrinkles in the 5 days immediately after the first treatment session, and at the 6-month assessment point, with the condition of the wrinkles pretreatment set at the 100% baseline. The edema after the first treatment probably caused this transitory improvement due to the plumping up of the skin, but the edema subsided the wrinkles became deeper again, although the final assessment still showed some improvement compared with the baseline. The averaged patient assessment of the evolution of his or her erythema can be seen in Fig. 2B, showing complete evolution by postirradiation day 4. Fig. 2C shows the patient assessment of the improvement in his or her skin texture immediately after the first treatment, and at the 6-month assessment point. Unlike the other values, this one interestingly continued to increase as time passed.

The computer-based image extraction program was used to assess objectively the directionality of

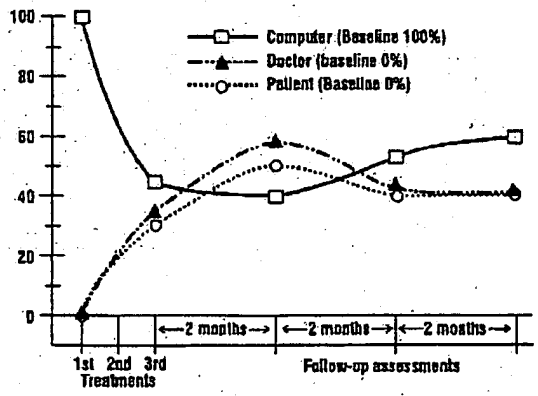


Figure 1 The subjective patient satisfaction index compared with the objective clinical indices from the clinician assessment and the computer image extraction program over the course of treatments and assessment points at 2, 4, and 6 months after the final treatment. Note that the patient and clinician assessments start from a pretreatment baseline of 0%, whereas the computer program baseline is 100%. Note also that the peak response occurs in all assessments at around 2 months after the last treatment, falls off thereafter, and plateaus at the 6-month assessment.

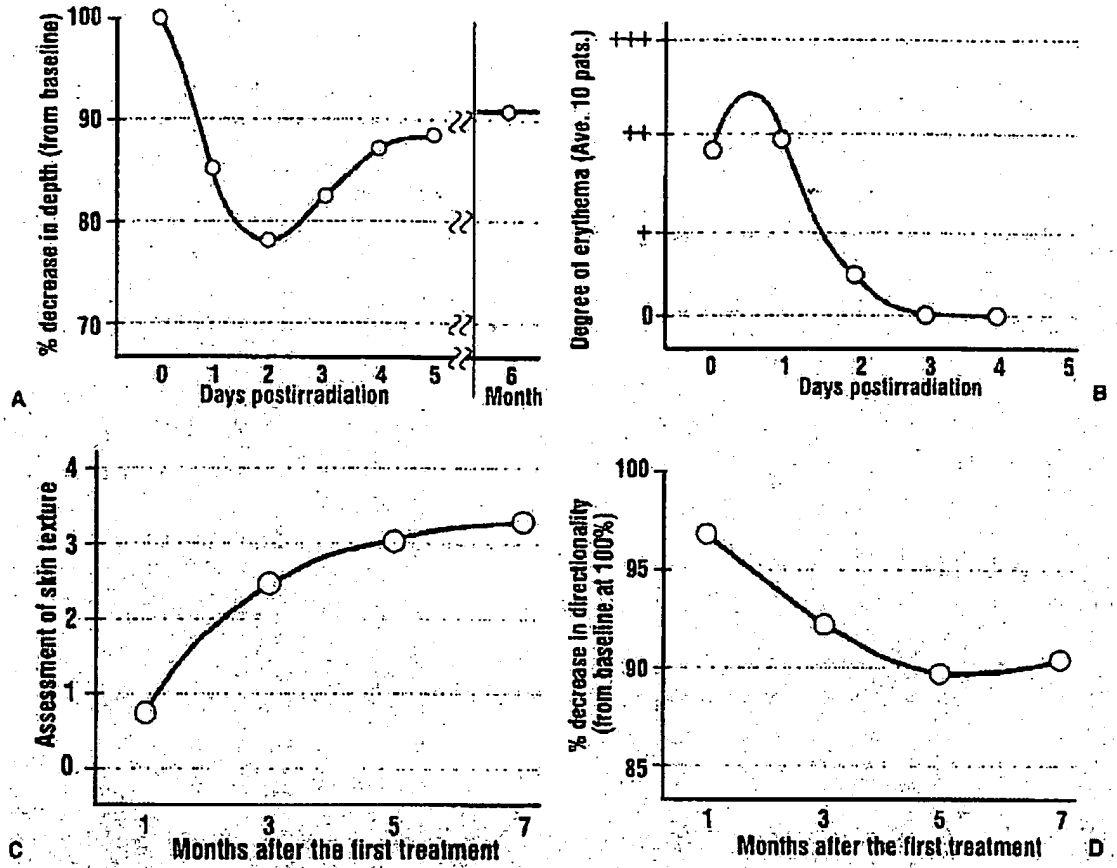


Figure 2 (A) Patient assessment of the improvement in the depth of their wrinkles in the 5 days immediately after the first treatment, and at the 6-month assessment point, with a pretreatment baseline of 100%. The greatest improvement was seen in the first 2 to 3 days, probably corresponding to the plumping up of the skin with mild edema. As that resolved the wrinkles deepened, so by the fifth posttreatment day, the level of improvement was almost the same as that from the 6-month assessment due to neocollagenesis. (B) This illustrates the progression of erythema after the first treatment session as assessed by the patients. Erythema had resolved in all patients by the fourth postirradiation day. (+ = no erythema; ++ = some erythema; +++ = noticeable erythema and ++++ = intense erythema. No patient reported intense erythema.) (C) Skin texture was also assessed by the patients at the third and final treatment session, then at the 2-, 4- and 6-month assessment points. Unlike the wrinkle improvement, the skin texture continues to improve over the assessment period, although a less dramatic improvement is seen from the 4-month assessment point, 5 months after the first treatment. (0 = no improvement, 1 = mild improvement, 2 = fair improvement, 3 = good improvement, and 4 = excellent improvement). (D) The computer image analysis program examined the directionality of wrinkles, seen as areas of depth lower than the surrounding tissue, and connecting perceived areas of depth linearly to represent wrinkles. The greater the directionality, the more apparent was the wrinkle, and vice versa. The pretreatment baseline is set at 100%.

wrinkles before the first treatment, after the third treatment (at 1 month), and then at 2, 4, and 6 months thereafter. Directionality means the tendency for the computer to recognize linear effects in the skin texture of the sample. The greater the directionality seen by the program, the more apparent was the wrinkle. An initial noticeable decrease in directionality gradually leveled out with the passage of time, as seen in Fig. 2D.

A typical example of the histological findings is seen in Fig. 3. In the preirradiation view (Fig. 3A), there was a typically elastotic dermis containing many interfibrillary spaces under a flattened epidermis with a disorganized stratum corneum. At the 2-month assessment point (i.e., 3 months after the first treatment), the appearance of the epidermis had thickened, the stratum corneum was more compact, and the dermis appeared much better organized, with a good layer of compact and linearly oriented dermal collagen coursing under the epidermis.

The 58-year-old skin type II female in Fig. 4 is a good representative example of the upper range of improvement following long-pulse Nd:YAG skin rejuvenation of her periocular region, seen before treatment (Fig. 4A) and at the 6-month assessment point after the final treatment (Fig. 4B). The wrinkles had improved slightly, but the skin texture was very good,

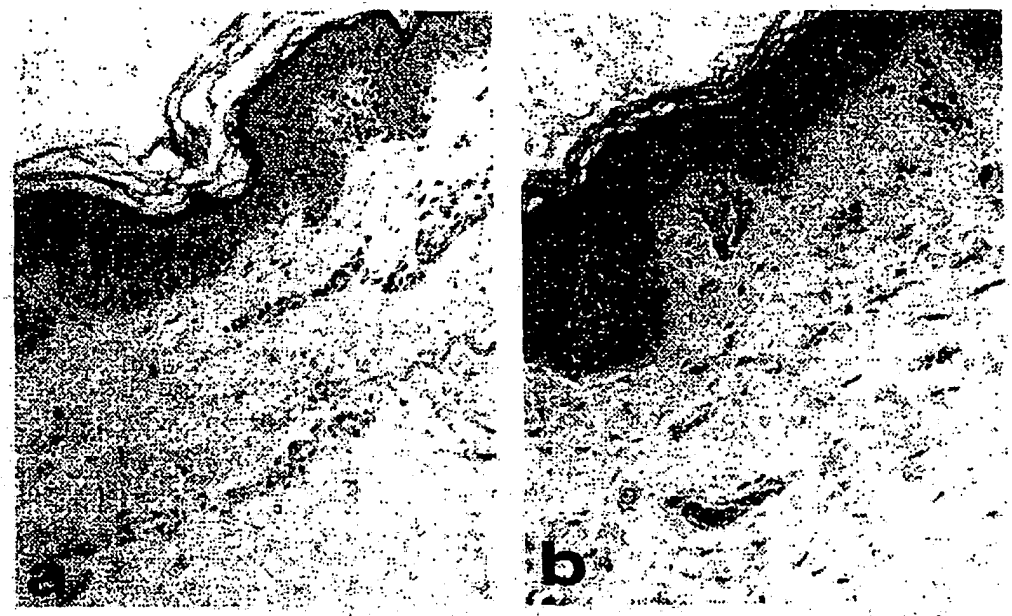


Figure 3 Representative histology (A) pretreatment and (B) 2 months after the final treatment (3 months after the first treatment). Skin, hematoxylin and eosin staining; original magnification ×200.

with removal of some small blood vessels in her upper cheek. Computer analysis shows pixel parameter detection and calculation to arrive at a percentage scoring of results. Percentage improvement is assigned after standardization of light condition and elimination of noise present in photos taken before and after (Figs. 4C and 4D).

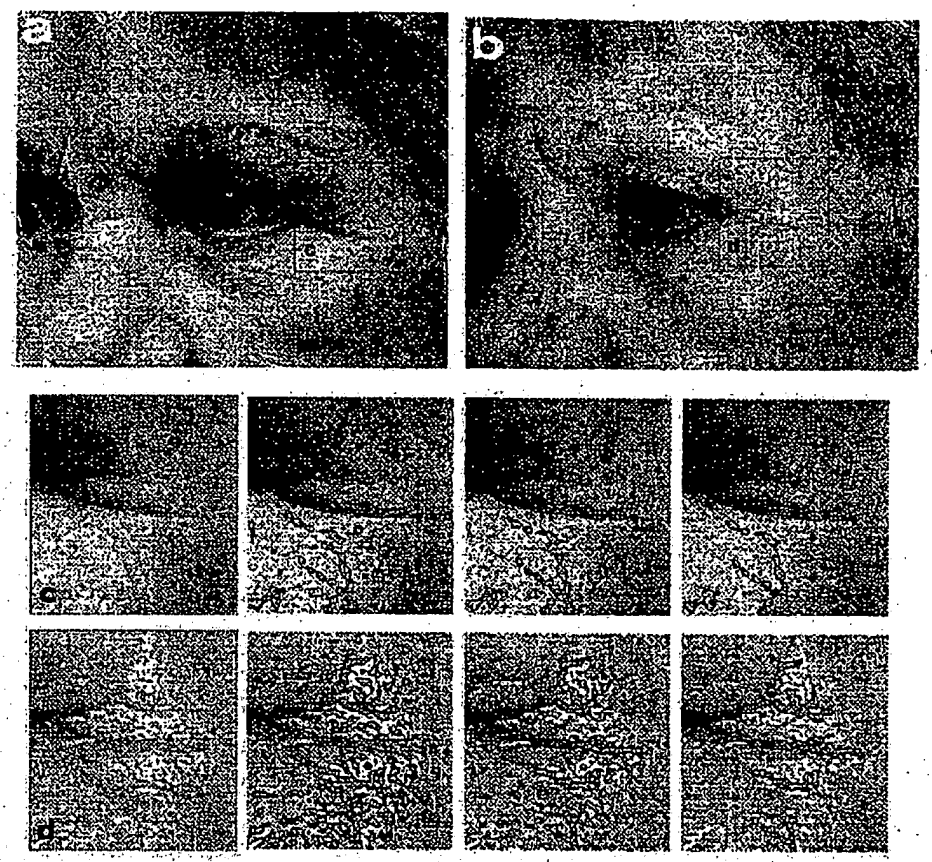
In Fig. 5A, a 61-year-old male is seen before treatment of his forehead wrinkles, and at the 6-month assessment point (Fig. 5B); some improvement could be seen in both the wrinkles and the skin texture.

DISCUSSION

Nonablative skin rejuvenation is based on the theory of delivering a useful packet of photothermal damage to the upper dermis under a cooled epidermis, thereby initiating the wound healing process under the biological protection of an intact epidermis and achieving the required collagen synthesis and remodeling.[13–15] The theory is very good and the dermal histology bears the theory out, as seen in Figs. 3A and 3B, but the results have been disappointing in practice from the standpoint of patient satisfaction. Some answers to this problem have come from combining wavelengths, usually one in the visible waveband followed by one from the near infrared; both systems normally use aggressive epidermal cooling.[16,17] However, this necessitates having two systems that remain rather expensive. If a system could be found that lessened the expense while maintaining the level of efficacy, it would be of benefit to clinicians and patients alike.

The long-pulsed Nd:YAG used in the current study operates at the wavelength of 1064 nm, the primary harmonic. This wavelength is mildly absorbed in water, but prefers proteinaceous targets such as blood vessels and the red blood cells within them, collagen, and melanin. Because the main target is protein with water as a secondary target, the heating effect in the target tissue absorbing the 1064-nm beam is nonspecific. In addition, because of the scattering effect of tissue on this wavelength, the area of greatest photon density, and thus the photothermal effect, is not at the surface of the tissue, but some 1 to 2 mm below the surface (Fig. 6), just at the region of interest for nonablative skin rejuvenation. The treatment technique, which involves using the laser in the noncontact mode and painting the beam over the target area, produces a mild heating in the skin, which can be controlled by positioning the handpiece further away from or nearer to the target tissue, to maintain the feeling of warmth that is the endpoint of thermal deposition in the dermis. The epidermis is not a very good target for this wavelength, and provided the heat in the dermis is not excessive, with no retrograde flow of secondary thermal damage, in our experience in the present study the epidermis could be spared with no cooling device required. Pain was not a major a factor in the study, with two patients reporting the treatment was very painful but bearable, and one patient reporting the treatment was painful. The additional pain did not correlate with better results, however.

The results of the study, on the other hand, show that the 6-month assessment patient SI and clinician and computer objective CI values were low (40%, 40%, and



Samples	c	d
Sample area (pixels)	15444	15444
Number of spots	3061	2176
Percentage (%)	19'82	14'09
Improvement (%)		28'91

Figure 4 Representative patient (58-year-old female, skin type II) (A) before treatment and (B) at the 6-month assessment point, 7 months after the first treatment. Improvement can be seen both in wrinkles and particularly in the skin texture condition, also in percentage terms detected by the computer program (C, D). For this assessment, an area of periocular wrinkles was selected and (C, D) shows the sequence of before and 6 months after the last treatment, with illustrations done by the Canny operator-based automatic edge detection process, to generate outlines of the wrinkles, which are then extracted as discrete images and shown in isolation. A reduction of the wrinkles is noticed from these images with an improvement of 28.91%. Refer to the text for details of the image analysis process.

50%, respectively) at a time when remodeling should still be producing good results for wrinkles. The short-term results, however, were interesting, as shown in Fig. 7, assessed during the three treatments from the clinical photography by the independent physician. This shows a fairly steep improvement after the first treatment, which leveled out after the second treatment and had plateaued by treatment 3. These data might suggest that a maintenance treatment or treatments sometime after the peaks in SI and CI values seen at the 2-month assessment stage might maintain the improvement, and further suggest that collagen synthesis increased swiftly after the first treatment, decreased by the third treatment, then increased again by the 2-month assessment. That might well be the appropriate time to give another treatment.

One obvious limitation to this study is the small number of patients from which meaningful percentages and statistics cannot be easily derived, but the results nevertheless show an apparent trend toward short-term collagen deposition with this single-modality approach. Additional treatments just at or after that 2-month peak

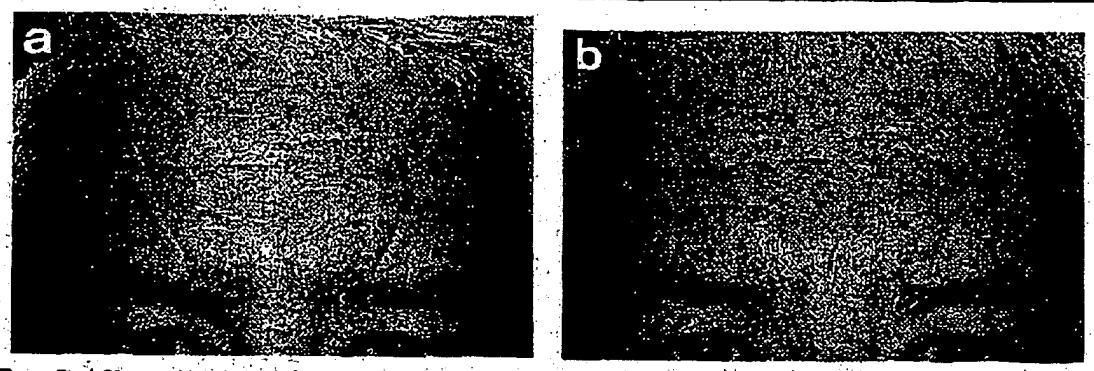


Figure 5 A 61-year-old male (A) before and (B) at the 6-month assessment after long-pulse Nd:YAG skin rejuvenation of his forehead. Subtle improvements in the wrinkles can be seen, with good results related to skin texture.

either with the system used in the present study alone, or with adjunctive epidermal and dermal care with appropriate cosmeceuticals, might have interesting results. Additional studies are merited in larger patient groups to elucidate if this would in fact be the case (as the manufacturers suggest) that this system can also be used with different parameters and techniques for hair removal and leg vein treatment, with obvious connotations for limiting increased treatment costs for the patient.

CONCLUSIONS

The results of the present single-modality study on long-pulsed Nd:YAG nonablative skin rejuvenation at the 6-month assessment point demonstrate that treatment does enhance skin rejuvenation. However, results were not maintained over time once treatment sessions had ceased, as is shown both from the subjective and objective analyses. The short-term improvement and the peak of improvement at the 2-month assessment were interesting, and indicate the possible amelioration of the longer term results with the addition of complementary maintenance treatments or repeated laser sessions.

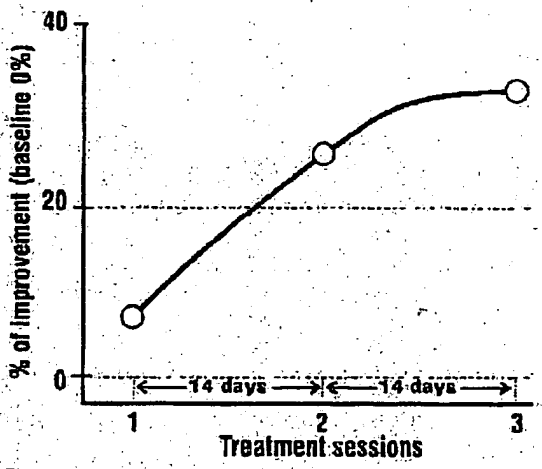


Figure 7 The interesting rapid improvement of wrinkles during the three treatments from a 0% baseline assessed by the clinician from the digital photography, reaching an objective clinical index (CI) of 34% after the third session (see also Fig. 1).

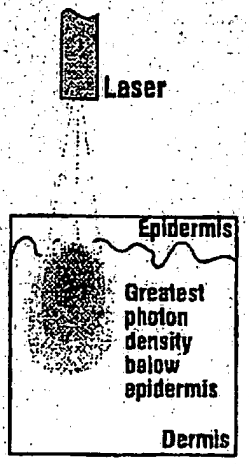


Figure 6 Schematic illustration of beam pattern of a 1064-nm Nd:YAG beam in tissue, showing the hot spot that exists under the epidermis instead of on the surface, due to the scattering pattern of this wavelength and its photoacceptors, namely protein and, to a lesser extent, water.

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